

## **CHEMICALLY DEFINED MEDIUM FOR CULTURED MAMMALIAN CELLS**

### **Field of the Invention:**

This application is based in part on, and claims priority to, U.S. Provisional 60/268,849 filed February 15, 2001, of which is entirely incorporated herein by reference.

The present invention in the field of biotechnology, relates to methods and compositions for providing chemically defined media for growth of cultured mammalian cells for production of commercially useful amounts of expressed proteins.

### **Background of the Invention:**

Bovine serum is commonly used in mammalian cell culture to promote cell growth and protein production. Since serum is expensive, non-defined animal materials such as primatone and albumin have been used as serum replacements. However, the quality of these non-defined animal proteins varies from batch to batch and consistent cell growth in these media is difficult to achieve. Moreover, pathogens such as prions and viruses have been identified as potential infectious agents (Balter, M. 2000, Kozak et al. 1996) that may reside in those animal derived products. Many regulations now strongly address these concerns about using serum or non-defined animal proteins in mammalian cells.

To support the growth of animal cells, a variety of components are essential to be included in the culture media. For example, glutamine and glucose are basic energy sources that support animal cell growth. Breakdown of these compounds provides resources for energy-generating pathways, the TCA cycle and glycolysis. The byproducts of these pathways are also the building blocks or sources for bio polymer synthesis (Petch and Bulter 1994). In addition, vitamins, amino acids and growth factors are also essential for robust cell growth by either suppressing the cascade of the suicide pathway known as apoptosis or by promoting the progression of the cell cycle so that cells may replicate (Franek F. 1994, Murakami et al. 1982, Mastrangelo et al. 1999, Xie and Wang, 1996, Muhamed Al-Rubeai 1998 ).

Trace elements are also important for the growth of animal cells. Ham and McKeehan (1979) noticed that adding trace elements, such as Zinc, iron, selenium, copper, molybdenum, and manganese, etc., was important for cloning and continuous passage of animal cells in stringent

conditions of serum-free media. Regardless, the importance of supplementing trace elements in the media for animal cells has not been well addressed (Schneider 1989, Merten and Litwin 1991). This may be due to the assumption that trace elements existed as contaminated impurities within serum or non-defined animal derived materials already.

Accordingly, there is also a need to provide chemically defined media for cell culture and/or production of heterologous proteins in commercially useful amounts.

### **Summary of Invention:**

The present invention provides chemically defined media (CDF) formulations and methods that provide certain compounds, amino acids, lipids, carbohydrates, trace elements and/or vitamins that provide a chemically defined media that excludes the use of non-defined animal derived raw materials (e.g., but not limited to, primatone, albumin and Excyte™, as well as other similar materials derived from serum or other animal derived proteins or products). Such media compositions and formulations of the present invention allow the growth of myeloma and other cell cultures to provide commercially useful amounts of the desired proteins expressed in such cell cultures. Accordingly the present invention provides specific media, formulations and methods of making and using thereof, as well as proteins provided therefrom. The present invention provides media that provide one or more advantages of being chemically defined, better protein producing, commercially suitable, cost-effective, and/or pose reduced regulatory concerns for proteins produced in cell lines grown therein.

### **Description of the Drawings**

**Figure 1** shows by graphical representation that CDM media of the present invention can support high cell density up to  $4.5 \times 10^6$  cells/mL on Day 7.

**Figure 2** shows by graphical representation that specific productivity for CDM culture is at  $16\mu\text{g}/10^6$  cells/day.

**Figure 3** shows that at high cell density between  $4\text{-}5 \times 10^6$  cells/mL, IgG production reached above  $60 \mu\text{g/mL}$ .

**Figure 4** shows by graphical representation that Viability of the CDM spinner culture remained above 75% throughout the experiment.

Also included in Figures 1-4 are data of C168O in SFM8 without primatone, albumin and C168J in SFM8 as references for comparison.

### **Detailed Description:**

The present invention provides media formulations and methods that provide a chemically defined media that provides advantages over known media, and which can be used for commercial production of mammalian cell-cultured proteins. The present invention also provides a chemically defined media (CDM) comprising novel components, as well as, or optionally further comprising, at least one of specified amino acids, lipids, carbohydrates, trace elements, vitamins, compounds and/or proteins, as described and enabled herein, in combination with what is known in the art.

The present invention avoids one or more problems associated with media that contains animal derived, or non-defined animal derived, components (e.g., but not limited to, primatone, albumin and excyte, as well as other similar materials derived from serum or other animal proteins in recombinant, synthetic or purified form).

Accordingly, chemically defined media (CDM) compositions and formulations of the present invention allow the growth of myeloma and other cell cultures to provide commercially useful amounts of the desired proteins expressed in such cell cultures. The present invention thus provides specific media formulations that are chemically defined, cost-effective, and pose reduced regulatory concerns compared to known media that comprise animal-derived materials that are not completely defined, or known chemically defined media.

Media of the present invention includes the substitution of specified components, without the use of animal derived proteins. In a preferred embodiment, media of the present invention comprises specified components, e.g., but not limited to, trace elements and vitamins, the media termed "chemically defined media" (CDM). Media of the present invention provides utility and improvements, including, but not limited to, at least one of suitable high cell density growth, improved growth rate, improved growth in scale up, improved viability, improved viability in scale up, improved protein yield, improved protein yield in scale up, and the like.

Suitable cell lines that can be used according to the present invention include any transformed or immortalized mammalian cell line. Such cell lines can include myeloma cell lines, such as Sp2/0, NSO, NS1, CHO, BHK, Ag653, P3X63Ag8.653 cells (ATCC Accession Number

CRL-1580) and SP2/0-Ag14 cells (ATCC Accession Number CRL-1851), COS-1 (e.g., ATCC CRL 1650), COS-7 (e.g., ATCC CRL-1651), HEK293, BHK21 (e.g., ATCC CRL-10), CHO (e.g., ATCC CRL 1610, CHO DXB-11, CHO DG44), BSC-1 (e.g., ATCC CRL-26) cell lines, HepG2 cells, P3X63Ag8.653, 293 cells, HeLa cells, NIH 3T3, COS-1, COS-7, NIH 273, and the like, or any cells derived therefrom, including cell fusions of the above, such as to protein producing cells, such as B-cells, antibody producing cells, isolated or cloned spleen or lymph node cells, and the like. A preferred cell line is derived from Sp2/0 and is designated C463A, as described herein.

Cell lines, such as those presented herein, can be adapted to a chemically defined media according to the present invention, using known techniques and/or as described herein. Such methods can take from 1-30 days, or up to several months, depending on the particular cell line and media formulation used. However, adaption of mammalian cells to grow in chemically defined media of the present invention is unexpectedly found to occur in significantly shorter times than known defined or undefined media.

At least one formulation of media of the present invention is unexpectedly discovered to provide at least one of several advantages over known media, including at least one of: (1) supporting improved or robust growth and protein or antibody production from various mammalian cell lines; (2) facilitated adaptation for protein producing cell lines; (3) cost-effective media components, as compared to known components, such as bovine serum and excyte, which do not need to be and are not included; and/or (4) better suitability for regulatory approval as the media components are at least one of better defined, do not include animal derived proteins or other products, and do not contain or potentially contain infectious agents.

The use of this medium in cell culture technologies, such as but not limited to culture dishes, culture plates, culture bottles, suspension culture, spin filter suspension culture, bioreactors, perfusion type bioreactors, mammalian cell fermentation culture, or any other suitable type of cell culture, is also included in the present invention.

A media formulation of the present invention includes at least one of specified buffers, salts, carbohydrates, vitamins, proteins, amino acids, lipids, trace elements, minerals, and the like as described herein in combination with what is known in the art.

The media preferably comprises, in addition to known mammalian or hybridoma cell culture components without undefined protein-or animal-derived components, at least one or more

of ammonium metavanadate, cadmium chloride, chromic potassium sulfate, ferric citrate, germanium dioxide, molybdic acid, salt or ammonium salt, nickel sulfate, zirconium chloride and/or hydrocortisone, or any suitable form, salt, halide, hydrate, solution, suspension, emulsion, or colloid thereof, powder and the like. In preferred embodiments, the media comprises, in addition to known components, at least one, two, three, four, five, six, seven, eight, or nine of the above components.

Non-limiting examples of such buffers and include at least one of MOPS, sodium phosphate, potassium phosphate, HEPES, and other known buffers. Salts included in such buffers include, but are not limited to sodium chloride, potassium chloride, and the like. Non-limiting examples are presented as one or more of the salts, as hydrous, anhydrous or other salt form, in the following table:

Component	g/L
<b>INORGANIC SALTS</b>	
AlCl <sub>3</sub> •6H <sub>2</sub> O	0.0000001-0.00001
NH <sub>4</sub> VO <sub>3</sub>	0.00000006-0.000001
BaCl <sub>2</sub>	0.0000002-0.000001
CaCl <sub>2</sub> •2H <sub>2</sub> O	0.004-0.09
CoCl <sub>2</sub> •6H <sub>2</sub> O	0.0000002-0.00001
CrK(SO <sub>4</sub> ) <sub>2</sub>	0.0000001-0.00001
CuSO <sub>4</sub> •5H <sub>2</sub> O	0.0000005-0.00001
FeSO <sub>4</sub> •7H <sub>2</sub> O	0.000001-0.0001
GeO <sub>2</sub>	0.00000005-0.000001
LiCl	0.001-0.1
MgCl•6H <sub>2</sub> O	0.01-1.0
MnCl(anhyd)	0.00000001-0.000001
Na <sub>2</sub> Moo <sub>4</sub> •2H <sub>2</sub> O	0.00000001-0.000001
NiNo <sub>3</sub> •6H <sub>2</sub> O	0.00000002-0.000001
KBr	0.00000001-0.000001
KCl	0.01-1.0
KI	0.00000001-0.000001
RbCl	0.000000001-0.0000001
AgCl	0.0000000004-0.0000001
NaHCO <sub>3</sub>	0.0000001-0.00001
NaCl	0.1-50
NaF	0.0000004-0.00001
Na <sub>2</sub> HPO <sub>4</sub> (anhyd)	0.01-5
Na <sub>2</sub> Seo <sub>3</sub>	0.000003-0.0001
SnCl <sub>2</sub> •2H <sub>2</sub> O	0.00000001-0.000001
TiO <sub>2</sub>	0.0000001-0.0001
ZnSo <sub>4</sub> •7H <sub>2</sub> O	0.000008-0.0001

Such carbohydrates include, but are not limited to, glucose (dextrose), fructose, mannose, galactose, and any other suitable monosaccharide, disaccharide, polysaccharide, polymer, carbohydrate and the like. Non-limiting examples of amounts include 0.0000001-100 g/L for one or more carbohydrate components.

Such vitamins and co-factors can include, but are not limited to, biotin, ascorbic acid, pantothenate, choline, folate, inositol, niacin, niacinamide, pyridoxal, riboflavin, thiamine, cyanocobalamin, L-ascorbic acid and salts, D-biotin, calciferol, choline, cocarboxylase, coenzyme A, 2-deoxyadenosine, 2-deoxyguanosine, 2-deoxycytidine, ergocalciferol, flavin adenosine dinucleotide, FAD, folic acid, D-glucuronic acid, lactone, D-glucuronic acid, glutathione, myo-inositol, mammalian recombinant insulin, menadione, 5'methylcytosine, niacinamide, NADP, NAD, nicotinic acid, oxalacetic acid, p-amino benzoic acid, D-pantothenic acid, pyroxidal, pyroxidine, retinol acetate, riboflavin,  $\alpha$ -tocopherol, thiamine, thymidine, UMP, UDP, UTP, AMP, ADP., ATP, GMP, GDP, GTP, CMP, CDP, CTP, TMP, TDP, TTP, vitamin B12, and the like, in any suitable form, such as salt, acid, base, and the like.

Such proteins or amino acids include, but are not limited to, alanine, arginine, asparagine, aspartate, cysteine, cystine, glutamine, glutamate, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, and salts or other derivatives thereof. Alternatively, such amino acids include at least one of L- $\alpha$ -amino-n-butyric acid, L-alanine, L-arginine, L-aspartic acid, L-asparagine, L-cysteine, L-citrulline, L-cysteine, D-glucosamine, L-glutamic acid, L-glutamine, L-glycine, L-histidine, hydroxy-L-Proline, L-isoleucine, L-leucine, L-lysine, L-methionine, L-ornithine, L-phenylalanine, L-proline, L-serine, taurine, L-threonine, L-tryptophan, L-tyrosine, L-valine, and the like, as well as salts, hydrates, hydrides, acids, bases thereof and the like.

Such trace elements and minerals include, but are not limited to, salts (e.g., chlorides, iodides, bromides, fluorides, sodium or potassium salts, phosphates, salts, and the like), acids (e.g., acetates, sulfates, sulfides, nitrates, nitrides, dioxides, and the like), bases (e.g., NaOH, KOH, and the like), of magnesium, potassium, sodium, calcium, and the like, such as sodium acetate, sodium chloride, sodium phosphate, selenium, aluminum, ammonium metavanadate, barium, cadmium, cobalt chloride, chromic potassium sulfate, cupric sulfate, ferric citrate, germanium dioxide, lithium chloride, magnesium chloride, manganese chloride, molybdic acid, nickel nitrate, potassium bromide, potassium iodide, rubidium chloride, silver chloride, sodium fluoride, stannous

chloride, sodium silicate, tin chloride tin chloride, titanium chloride, zinc sulfate, zirconium oxychloride, and the like, and salts thereof.

As a further non-limiting example, a formulation of CDM media of the present invention comprises: sodium chloride, 3-5 g/L; potassium chloride, 0.2-0.4 g/L; , HEPES, 5-7 g/L; glucose (dextrose), 3.5-5.5 g/L; biotin, 0.000005-0.000025 g/L; ascorbic acid, 0.002-0.004; pantothenate, 0.002-0.006 g/L; choline, 0.002-0.006 g/L; folate, 0.002-0.006 g/L; inositol, 0.005-0.02 g/L; niacinamide, 0.002-0.006 g/L; pyridoxal, 0.002-0.006 g/L; riboflavin, 0.0002-0.0006 g/L; thiamine, 0.002-0.006 g/L; cyanocobalamin, 0.000005-0.000025 g/L; oxaloacetic acid, 0.1-0.4 g/L; alanine, 0.015-0.035 g/L; asparagine, 0.01-0.035 g/L; arginine, 0.06-0.10 g/L; aspartate, 0.02-0.04 g/L; cysteine, 0.3-0.5 g/L; cystine, 0.05-0.2 g/L; glutamine, 0.8-1.5 g/L; glutamate, 0.06-0.09 g/L; glycine, 0.02-0.04 g/L; histidine, 0.03-0.05 g/L; isoleucine, 0.05-0.25 g/L; leucine, 0.05-0.25 g/L; lysine, 0.05-0.25 g/L; methionine, 0.02-0.04 g/L; phenylalanine, 0.055-0.075. proline, 0.03-0.05 g/L; serine, 0.03-0.055 g/L; threonine, 0.07-0.15 g/L; tryptophan, 0.005-0.025 g/L; tyrosine, 0.05-0.15 g/L; valine, sodium selenate, 0.0000005-0.000060; magnesium sulfate, 0.05-0.2 g/L; potassium chloride, 0.15-0.45 g/L; sodium phosphate, 0.075-0.2 g/L; potassium nitrate, 0.00005-0.00009 g/L; calcium chloride, 0.08-0.25 g/L; sodium pyruvate 0.05-0.4 g/L; insulin, 0.05-2 g/L; hydrocortisone, 20-80 µg/L; linoleic acid, 1-100 mg/L; ethanolamine, 5-25 µg/L; sodium bicarbonate, 1-5 g/L; APO transferrin or ferric citrate, 1-10 mg/L; Pluronic F68, 0.2-2 g/L; sodium hydroxide, 0.3-0.9 g/L; mycophenolic acid, 0.1-2 mg/L; hypoxanthine, 2-5 mg/L; xanthine; 10-200 mg/L; sodium bicarbonate 1.5-4.5 g/L.

Known serum free hybridoma media that can be modified to provide the media of the present invention include, but are not limited to, e.g., Sigma/Aldrich product numbers S2772, S2897 and S8284 ([www.sigma-aldrich.com](http://www.sigma-aldrich.com)); similar known serum free media include those from Life Technologies, Rockville, MD ([www.lifetech.com](http://www.lifetech.com)) and JRH Biosciences, Lenexa, KS ([www.jrhbio.com](http://www.jrhbio.com)). For example, known serum free hybridoma cell cultures can include HEPES or MOPS, sodium bicarbonate, L-glutamine, cholesterol, insulin, BSA, transferrin or ferric citrate, in addition to other serum free mammalian cell culture components. See, e.g., SIGMA catalog, 1998, pp 1776-1777, 1677-1704, 1715-1755, 1795-1847, entirely incorporated herein by reference. Non-limiting examples of known serum free media that can be modified to provide CDM of the present invention include, but are not limited to, sigma media product numbers S2772, S2897 and S8284, as follows:

SIGMA Prod. #	S 2897	S 8284	S2772
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Component	g/L	g/L	g/L
<b>INORGANIC SALTS</b>			
AlCl <sub>3</sub> •6H <sub>2</sub> O	0.000001	0.000001	0.000001
NH <sub>4</sub> VO <sub>3</sub>	0.0000006	0.0000006	0.0000006
BaCl <sub>2</sub>	0.000002	0.000002	0.000002
CaCl <sub>2</sub> •2H <sub>2</sub> O	0.0441	0.0441	0.0441
CoCl <sub>2</sub> •6H <sub>2</sub> O	0.000002	0.000002	0.000002
CrK(SO <sub>4</sub> ) <sub>2</sub>	0.000001	0.000001	0.000001
CuSO <sub>4</sub> •5H <sub>2</sub> O	0.0000051	0.0000051	0.0000051
FeSO <sub>4</sub> •7H <sub>2</sub> O	0.000834	0.000834	0.000834
GeO <sub>2</sub>	0.0000005	0.0000005	0.0000005
LiCl	0.01	0.01	0.01
MgCl•6H <sub>2</sub> O	0.123	0.123	0.123
MnCl(anhyd)	0.0000001	0.0000001	0.0000001
Na <sub>2</sub> MoO <sub>4</sub> •2H <sub>2</sub> O	0.0000001	0.0000001	0.0000001
NiNO <sub>3</sub> •6H <sub>2</sub> O	0.0000002	0.0000002	0.0000002
KBr	0.0000001	0.0000001	0.0000001
KCl	0.224	0.224	0.224
KI	0.0000001	0.0000001	0.0000001
RbCl	0.00000001	0.00000001	0.00000001
AgCl	0.0000000044	0.0000000044	0.0000000044
NaHCO <sub>3</sub>	—	2.25	2.25
NaCl	7.599	7.599	7.599
NaF	0.000004	0.000004	0.000004
Na <sub>2</sub> HPO <sub>4</sub> (anhyd)	0.39739	0.39739	0.39739
Na <sub>2</sub> SeO <sub>3</sub>	0.00003	0.00003	0.00003
SnCl <sub>2</sub> •2H <sub>2</sub> O	0.0000001	0.0000001	0.0000001
TiO <sub>2</sub>	0.000001	0.000001	0.000001
ZnSO <sub>4</sub> •7H <sub>2</sub> O	0.000863	0.000863	0.000863
<b>AMINO ACIDS</b>			
L-Alanine	0.009	0.009	0.009
L-Arginine	0.211	0.211	0.211
L-Asparagine•H <sub>2</sub> O	0.03401	0.03401	0.03401
L-Aspartic Acid	0.0133	0.0133	0.0133
L-Citrulline	0.005	0.005	0.005
L-Cysteine•HCl•H <sub>2</sub> O	0.035	0.035	0.035
L-Glutamic Acid	0.0147	0.0147	0.0147
L-Glutamine	0.396	0.396	0.396
Glycine	0.00751	0.00751	0.00751
L-Histidine•HCl•H <sub>2</sub> O	0.071	0.071	0.071
L-Isoleucine	0.164	0.164	0.164
L-Leucine	0.133	0.133	0.133
L-Lysine•HCl	0.109	0.109	0.109
L-Methionine	0.015	0.015	0.015
L-Ornithine	0.008	0.008	0.008
L-Phenylalanine	0.055	0.055	0.055

See, e.g., Ham et al., Proc. Natl. Acad. Sci. USA 53:288-193 (1965); Myoken et al., In Vitro 25:477-480 (1989).



More preferably, the media further comprises at least one selected from the group consisting of buffers, salts, carbohydrates, amino acids, lipids, vitamins, co-factors, and the like in suitable form. Suitable media that can be modified according to the present invention can include one or more or a combination of Iscove's modified media, Dulbecco's Modified Eagle Medium, Ham's F-12 media, e.g., as provided by SIGMA, LIFE TECHNOLOGIES OR JRH BIOSCIENCES, as listed above. Non-limiting examples, include, but are not limited to:

**Iscove's Modified Media: (Sigma I 2510, I 7633, I 2762, I 3390):**

SIGMA Prod. Num.	I 2510, I 7633	I 2762, I 3390
COMPONENT	g/L	g/L
<b>INORG. SALTS</b>		
CaCl <sub>2</sub> •2H <sub>2</sub> O	0.219	0.219
MgSO <sub>4</sub> (anhyd)	0.09767	0.09767
KCl	0.33	0.33
KN03	0.000076	0.000076
NaHC03	—	3.024
KCl	4.505	4.505
NaH2P04 (anhyd.)	0.109	0.109
Na2Se03	0.000017	0.000017
<b>AMINO ACIDS</b>		
Alanine	0.025	0.025
L-Arginine•HCl	0.084	0.084
L-Asparagine•H <sub>2</sub> O	0.0284	0.0284
L-Aspartic Acid	0.03	0.03
L-Cystine•2HCl	0.09124	0.09124
L-Glutamic Acid	0.075	0.075
L-Glutamine	0.584	—
Glycine	0.03	0.03
L-Histidine•HCl•H <sub>2</sub> O	0.042	0.042
L-Isoleucine	0.105	0.105
L-Leucine	0.105	0.105
L-Lysine•HCl	0.146	0.146
L-Methionine	0.03	0.03
L-Phenylalanine	0.066	0.066
L-Proline	0.04	0.04
L-Serine	0.042	0.042
L-Threonine	0.095	0.095
L-Tryptophan	0.016	0.016
L-Tyrosine•2Na•2H <sub>2</sub> O	0.10379	0.10379
L-Valine	0.094	0.094
<b>VITAMINS</b>		
D-Biotin	0.000013	0.000013
Choline Chloride	0.004	0.004
Folic Acid	0.004	0.004
myo-Inositol	0.0072	0.0072
Niacinamide	0.004	0.004
D-Pantothenic Acid•½Ca	0.0004	0.004
Pyridoxal•HCl	0.004	0.004
Riboflavin	0.0004	0.0004
Thiamine•HCl	0.004	0.004
Vitamin B12	0.000013	0.000013
<b>OTHER</b>		

D-Glucose	4.5	4.5
HEPES	5.958	5.958
Phenol Red•Na	0.016	0.016
Pyruvic Acid•Na	0.11	0.11
ADD		
NaHCO <sub>3</sub>	3.024	—
L-Glutamine	—	0.584
Grams of powder required to prepare 1 L	17.7	N/A

See, e.g., Iscove et al., J. Exp. Med. 147:923-933 (1978); Iscove, et al., Exp. Cell Res. 126:121-126 (1980).

**Dulbecco's Modified Eagle's Medium (e.g., Sigma D0422, D 1152, D 2429, D 2554, D2902, D 3656, D 5030, D5280, D5523).**

SIGMA Prod #	D0422	D1152	D2429	D2554	D2902	D3656	D 5030	D5280	D 5523
COMPONENT	g/L	g/L	g/L	g/L	g/L	g/L	g/L	g/L	g/L
<b>INORGANIC</b>	<b>SALTS</b>								
CaCl <sub>2</sub> •2H <sub>2</sub> O	0.265	0.265	2.65	2.65	0.265	0.265	0.265	0.265	0.265
Fe(NO <sub>3</sub> ) <sub>3</sub> •9H <sub>2</sub> O	0.0001	0.0001	0.001	0.001	0.0001	0.0001	0.0001	0.0001	0.0001
MgSO <sub>4</sub>	0.09767	0.09767	0.9767	0.9767	0.09767	0.09767	0.09767	0.09767	0.09767
KCl	0.4	0.4	4.0	4.0	0.4	0.4	0.4	0.4	0.4
NaHCO <sub>3</sub>	3.7	—	—	—	—	—	—	—	—
NaCl	6.4	4.4	64.0	64.0	6.4	6.4	6.4	6.4	6.4
NaH <sub>2</sub> PO <sub>4</sub>	0.109	0.109	1.09	1.09	0.109	—	0.109	0.109	0.109
Succinic Acid	—	—	—	—	—	—	0.075	—	—
Sodium Succinate	—	—	—	—	—	—	0.1	—	—
<b>AMINO</b>	<b>ACIDS</b>								
L-Arginine•HCl	0.84	0.084	0.84	0.84	0.084	0.084	0.084	0.084	0.084
L-Cystine•2HCl	—	0.0626	0.626	0.626	0.0626	0.0626	0.0626	0.0626	0.0626
L-Glutamine	0.03	0.584	0.30	0.30	0.584	0.584	—	—	0.584
Glycine	0.042	0.030	0.42	0.42	0.030	0.030	0.030	0.030	0.030
L-Histidine•HCl•H <sub>2</sub> O	0.105	0.042	1.05	1.05	0.042	0.042	0.042	0.042	0.042
L-Isoleucine	0.105	0.105	1.05	1.05	0.105	0.105	0.105	0.105	0.105
L-Leucine	1.46	0.105	1.46	1.46	0.105	0.105	0.105	0.105	0.105
L-Lysine•HCl	—	0.146	0.30	0.30	0.146	0.146	0.146	0.146	0.146
L-Methionine	0.066	0.030	0.66	0.66	0.030	0.030	0.030	0.030	0.030
L-Phenylalanine	0.042	0.066	0.42	0.42	0.066	0.066	0.066	0.066	0.066
L-Serine	0.095	0.042	0.95	0.95	0.042	0.042	0.042	0.042	0.042
L-Threonine	0.016	0.095	0.16	0.16	0.095	0.095	0.095	0.095	0.095
L-Tryptophan	0.016	—	—	0.016	0.016	0.016	0.016	0.016	—
L-Tyrosine (free base)	0.10379	—	—	1.0379	1.0379	—	—	0.072	—
L-Tyrosine•2Na•2H <sub>2</sub> O	0.10379	0.10379	0.10379	0.10379	—	0.10379	—	—	—
L-Valine	0.094	0.094	0.94	0.94	0.094	0.094	0.094	0.094	0.094
<b>VITAMINS</b>									
Choline Bitartrate	0.004	—	0.04	0.04	—	—	—	0.0072	—
Choline Chloride	0.004	0.004	—	—	0.004	0.004	0.004	—	0.004
Folic Acid	0.0072	0.004	0.072	0.072	0.004	0.004	0.004	0.004	0.004
myo-Inositol	0.004	0.0072	0.04	0.04	0.0072	0.0072	0.0072	0.0072	0.0072

Niacinamide	0.004	0.004	0.04	0.04	0.004	0.004	0.004	0.004	0.004
D-Pantothenic Acid•½Ca	0.004	0.004	—	—	0.004	0.004	0.004	0.004	0.004
Pyridoxal•HCl	—	0.004	0.04	0.04	0.004	0.004	0.004	0.004	0.004
Pyridoxine•HCl	0.0004	—	0.004	0.004	—	—	—	—	—
Riboflavin	0.004	0.0004	0.04	0.04	0.0004	0.0004	0.0004	0.0004	0.0004
Thiamine•HCl	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
<b>OTHER</b>									
D-Glucose	4.5	4.5	10.0	45.0	1.0	4.5	—	1.0	—
HEPES	—	5.958	—	—	—	—	—	—	0.0159
Phenol Red•Na	0.0159	0.0159	0.159	0.159	-----	0.0159	-----	0.0093	0.11
Pyruvic Acid•Na	0.11	-----	1.1	1.1	0.11	-----	—	0.11	—
<b>ADD</b>									
Glucose	—	—	—	—	—	1.0	-----	—	—
L-Glutamine	0.584	—	0.584	0.584	-----	-----	0.584	0.584	-----
L-Cystine•2HCl	—	—	—	—	—	—	—	—	—
L-Leucine	—	—	—	—	—	—	—	—	—
L-Lysine•HCl	—	—	—	—	—	—	—	—	—
L-Methionine	—	—	—	—	—	—	—	—	—
NaHCO <sub>3</sub>	—	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7
NaH <sub>2</sub> PO <sub>4</sub>	—	—	—	—	—	0.109	—	—	—
Phenol Red•Na	-----	—	—	—	—	—	—	—	—
Pyruvic Acid•Na	-----	—	—	—	—	—	—	—	—
<b>Grams of powder to prepare 1 L</b>	N/A	17.4	N/A	N/A	N/A	N/A	N/A	N/A	10.0

See, e.g., Dulbecco and Freeman, Virology 8:396-397 (1959); Smith et al., J.D., Freeman, G., Vogt, M. and Dulbecco, R. (1960). Virology 12:185-196 (1960); Morton, In Vitro 6:89 (1970); Rutzky and Pumper, In Vitro 9:468 (1974).

**Ham's F-12/Dulbecco's Modified Eagle's Medium (e.g., Sigma D6905, D 8900, D 2906, D 9785, D6421)**

SIGMA Prod.#	D6905, D8900	D 2906	D 9785	D 6421
<b>COMPONENT</b>				
<b>INORGANIC SALTS</b>	<b>g/L</b>	<b>g/L</b>	<b>g/L</b>	<b>g/L</b>
CaCl <sub>2</sub> •2H <sub>2</sub> O	0.1545	0.1545	—	0.1545
CuSO <sub>4</sub> •5H <sub>2</sub> O	0.0000013	0.0000013	0.0000013	0.0000013
Fe(NO <sub>3</sub> ) <sub>3</sub> •9H <sub>2</sub> O	0.00005	0.00005	0.00005	0.00005
FeSO <sub>4</sub> •7H <sub>2</sub> O	0.000417	0.000417	0.000417	0.000417
MgCl <sub>2</sub> •6H <sub>2</sub> O	0.06120	0.0612	—	0.0612
MgSO <sub>4</sub>	0.04884	0.04884	—	0.04884
KCl	0.3118	0.3118	0.3118	0.3118
NaHCO <sub>3</sub>	—	—	—	1.2
NaCl	6.996	6.996	6.996	6.996
Na <sub>2</sub> HPO <sub>4</sub>	0.07102	0.07102	0.07102	0.07102
NaH <sub>2</sub> PO <sub>4</sub>	0.0543	0.0543	0.0543	0.0543
ZnSO <sub>4</sub> •7H <sub>2</sub> O	0.000432	0.000432	0.000432	0.000432
<b>AMINO ACIDS</b>				
L-Alanine	0.00445	0.00445	0.00445	0.0045
L-Arginine•HCl	0.1475	0.1475	0.1475	0.1475
L-Asparagine•H <sub>2</sub> O	0.0075	0.0075	0.0075	0.0075
L-Aspartic Acid	0.00665	0.00665	0.00665	0.00665

L-Cystine•HCl•H <sub>2</sub> O	0.01756	0.01756	0.01756	0.01756
L-Cysteine•2HCl	0.03129	0.03129	0.03129	0.03129
L-Glutamic Acid	0.00735	0.00735	0.00735	0.00735
L-Glutamine	0.365	0.365	—	—
Glycine	0.01875	0.01875	0.01875	0.01875
L-Histidine•HCl•H <sub>2</sub> O	0.03148	0.03148	0.03148	0.03148
L-Isoleucine	0.05447	0.05447	0.05447	0.5447
L-Leucine	0.05905	0.05905	—	0.05905
L-Lysine•HCl	0.09125	0.09125	—	0.09125
L-Methionine	0.01724	0.01724	—	0.01724
L-Phenylalanine	0.03548	0.03548	0.03548	0.03548
L-Proline	0.01725	0.01725	0.01725	0.01725
L-Serine	0.02625	0.02625	0.02625	0.02625
L-Threonine	0.05345	0.05345	0.05345	0.05345
L-Tryptophan	0.00902	0.00902	0.00902	0.00902
L-Tyrosine•2Na•2H <sub>2</sub> O	0.05579	0.05579	0.05579	0.05579
L-Valine	0.05285	0.05285	0.05285	0.05285
<b>VITAMINS</b>				
D-Biotin	0.0000035	0.0000035	0.0000035	0.0000035
Choline Chloride	0.00898	0.00898	0.00898	0.00898
Folic Acid	0.00265	0.00266	0.00266	0.00266
myo-Inositol	0.0126	0.0126	0.0126	0.0126
Niacinamide	0.00202	0.00202	0.00202	0.00202
D-Pantothenic Acid•½Ca	0.00224	0.00224	0.00224	0.00224
Pyridoxal•HCl	0.002	0.002	0.002	—
Pyridoxine•HCl	0.000031	0.000031	0.000031	0.002031
Riboflavin	0.000219	0.000219	0.000219	0.000219
Thiamine•HCl	0.00217	0.00217	0.00217	0.00217
Vitamin B-12	0.00068	0.00068	0.00068	0.00068

See, e.g., Barnes and Sato , *Analyt. Biochem.* 102:255-270 (1980).

Any suitable or desired protein for mammalian cell expression can be used in cell culture using media according to the present invention. Non-limiting examples of such proteins include, but are not limited to therapeutic or diagnostic proteins, such as eukaryotic or prokaryotic proteins. Preferred proteins can include, but art not limited to, cytokines, receptors, soluble receptors, interleukins, growth factors, and the like.

#### Citations

All publications or patents cited herein are entirely incorporated herein by reference as they show the state of the art at the time of the present invention and/or to provide description and enablement of the present invention. Publications refer to scientific or patent publications, or any other information available in any media format, including all

recorded, electronic or printed formats. The following references are entirely incorporated herein by reference: Ausubel, et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, NY (1987-1999); Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> Edition, Cold Spring Harbor, NY (1989); Harlow and Lane, Antibodies, a Laboratory Manual, Cold Spring Harbor, NY (1989); Colligan, et al., eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-1998); Colligan et al., Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-1999).

### **Examples:**

#### **EXAMPLE 1: Production of Protein in Chemically Defined Media of the Present Invention Using Adapted Cell lines**

An IgG protein producing myeloma cell line named C168O was not ideally suited for commercial production of IgG or for suitable regulatory approval, due to need for media components of known serum free media containing animal protein derived preparations that were not sufficiently defined or characterized, such as Excyte and others. This Excyte dependency was not able to be alleviated by adding chemically defined lipids or other components. However, when Excyte was removed and trace elements /vitamins were supplemented, a robust growth of C168O was achieved. This medium without primatone, albumin and excyte but supplemented with trace elements and vitamins is now called "CDM". A semi-batch culture of C168O in CDM medium showed that CDM medium was able to support both high cell density growth and high IgG production.

Another myeloma cell line called C463A is capable of growing in various commercial defined media. However, this growth was not ideally suited for commercial production of IgG or for suitable regulatory approval. C463A is derived from Sp2/0 and potentially can be used as a transfection host to develop commercially suitable cell lines. In semi-batch cultures, the cell density of C463A in CDM medium of the present invention routinely reached 6 to 7 million cells per milliliter (ml) compared with 3 to 4 million per ml in other tested defined media. The viability is similar amongst all tested media (80% to 90%). Apparently, CDM has the capability to support cell growth at a higher density than other chemically defined media.

To adapt cell lines derived from Sp2/0 cells in chemically defined media is a lengthy process. It usually takes several months to one year to obtain one. When CDM medium is used, we noticed that the length of time for adaptation was much shorter than that in other defined media. In one case, it took only a few weeks to obtain CDM culture compared to several months from previous experiences.

In summary, we find that trace elements and vitamins are essential for the growth of myeloma cells in the absence of bovine serum and non-defined animal derived materials. A chemically defined formulation was generated based on the addition of trace elements and vitamins to a suitable serum free media system. This formulation provides several advantages: 1. Supports robust growth and IgG or other protein production of various myeloma and other cell lines, 2. Easy adaptation for mammalian cells, e.g., Sp2/0-derived IgG or protein producing cell lines, 3. Cost-effective since expensive components, such as bovine serum and excyte, are excluded and 4. Regulatory-friendly since potentially infectious agents are eliminated.

The use of this medium in perfusion type bioreactors is or other types of cell culture can also be used according to the present invention.

#### **Formulation of CDM medium:**

The formulation of a CDM media of the present invention is provided as follows, e.g., Tables A-B. Table A1 shows the components added to make the media. Tables A2-A3 and B1-B4 show the listing of components for the additional formulations used in Table A1. The components are available from commercial sources, as individual components, or as custom formulations that can be ordered, e.g., from Sigma (St. Louis, Mo., USA), Aldrich (St. Louis, Mo., USA), JRH Biosciences (Lenexa, KS, USA), and the like.

#### **TABLES A1-A3:**

**TABLE A1  
NON-LIMITING EXAMPLE OF CDM MEDIA OF THE INVENTION**

Components	Final Concentration In Media	Physical Property	Storage Temp.
CM-2 (Part A, Table A2)	18.8 g/l	Powder	+2-8°C
CH-2 (Part B; Table A3)	10 ml/l	Liquid	-20°C

NaHCO <sub>3</sub>	3.02 g/l	Powder	Ambient
Bovine APO Transferrin or ferric citrate	5 mg/l	Stock solution	+2-8°C
		Powder	+2-8°C
Pluronic F68	0.8 g/l	Stock Solution	+2-8°C
		Powder	Ambient
		Powder	+2-8°C
NaOH	0.7 g/l	Stock Solution	Ambient
		Pellets	Ambient
Ethanolamine	10 or 20 mg/l	Stock Solution	-20°C
		Liquid	Ambient
Glutamine	0.29 g/l	Powder	Ambient
Mycophenolic acid	0.5 mg/l	Stock Solution WSS 2	+2-8°C
Hypoxanthine	2.5 mg/l		
Xanthine (MHX)	50 mg/l		
Hydrocortisone	20 µg/l	WSS 9	+2-8°C
Vitamins (Table B1)	1X	100X Liquid	+2-8°C
Trace Minerals 1 (Table B1)	1X	1000X Liquid	+2-8°C
Trace Minerals 2 (Table B2)	1X	1000X Liquid	+2-8°C
Trace Minerals 3 (Table B3)	1X	1000X Liquid	+2-8°C

Preparation Instructions: Add components in order listed above. The sodium hydroxide should be made the same day.

Note: Prior to pH adjustment, pH = 6.7 – 6.8. The density at liquid stock solutions are the same as water (p – 1 g/ml). Therefore, volume or weight can be used alternatively.

SI pH: 7.3 – 7.6

SI Osm: 305 – 368

**TABLE A2: CM-2 (Part A)**

Component	Final Conc. gm/L (Dry, 18.8 g total/L)
Sodium Chloride	4.505
Potassium Chloride	0.330

Sodium Phosphate Monobasic H <sub>2</sub> O	0.125
Magnesium Sulfate, Anhydrous	0.09767
Potassium Nitrate	0.000076
Sodium Selenite	0.0000173
Calcium Chloride, Anhydrous	0.165
L-Alanine	0.025
L-Asparagine H <sub>2</sub> O	0.0284
L-Arginine HCl	0.084
L-Aspartic Acid	0.030
L-Cysteine HCl H <sub>2</sub> O	0.4175
L-Cystine 2HCl	0.09124
L-Glutamic Acid	0.075
L-Glutamine	0.8763
Glycine	0.030
L-Histidine HCl H <sub>2</sub> O	0.042
L-Isoleucine	0.105
L-Leucine	0.105
L-Lysine HCl	0.146
L-Methionine	0.030
L-Phenylalanine	0.066
L-Proline	0.040
L-Serine	0.042
L-Threonine	0.095
L-Tryptophan	0.016
L-Tyrosine 2Na 2H <sub>2</sub> O	0.10379
L-Valine	0.094
Dextrose	4.500
Sodium Pyruvate	0.220
Biotin	0.000013
Ascorbic Acid	0.003
D-Ca Pantothenate	0.004
Choline Chloride	0.004
Folic Acid	0.004
i-Inositol	0.0072
Niacinamide	0.004
Pyridoxal HCl	0.004
Riboflavin	0.0004
Thiamine HCl	0.004
Cyanocobalamin	0.000013
Oxalacetic Acid	0.300
HEPES	5.958
	<b>18.7776193</b>



**TABLE A3: CH-2 (Part B) (100X)**

Component	100X: Amount/L	Final Conc.
Insulin	1.0 g	10 mg/L
Hydrocortisone	200 µg	2 µg/L
Linoleic Acid	500 mg	5 mg/L
Ethanolamine (1.02 mg/µl)	1020 g	10 mg/L
NaCl	8.5 g	85 mg/L

CDM medium is prepared according to Table A1 by adding components CH-2, parts A (18.8 gm/L, Table A2) and B (10 ml/L (100X), Table A3), followed by NaHCO<sub>3</sub> (3.02 g/L), Bovine APO transferrin or ferric citrate (5 mg/L), Pluronic F68 (0.8 g/L), NaOH (0.7 g/L), Ethanolamine (10µl/L), Glutamine (0.29 g/L), mycophenolic acid (0.5 mg/L), hypoxanthine (2.5 mg/L), xanthine (50 mg/L), hydrocortisone (20 µg/L), vitamins (100X, 10 ml/L, Table B1), trace minerals 1 (1000X, 0.33-1.0 ml/L, Table B2), trace minerals 2 (1000X, 0.33-1.0 ml/L, Table B3), trace minerals 3 (1000X, 0.33-1.0 ml/L, Table B4). In this example of CDM medium of the present invention, the working concentration of trace elements is 0.33-1.00X, and 1X for vitamins.

**TABLES B1-B4:****TABLE B1: Vitamin Solution (100X)**

Component	Final Concentration Added (1X)	Liquid 100X
	mg/L	mg/L
NaCl	85.00	8500.00
D-Calcium Pantothenate	1.00	100.00
Choline Chloride	1.00	100.00
Folic Acid	1.00	100.00
i-Inositol	2.00	200.00
Nicotinamide	1.00	100.00

Pyridoxine – HCl	1.00	100.00
Riboflavin	0.1	10.00
Thiamine – HCl	1.00	100.00

**TABLE B2: Trace Metals 1, 1000X**

COMPONENT	Final Conc. Added 0.33X	Final Conc. Added 1X	Liquid 1000X
	µg/L	µg/L	mg/L
<b>CuSO<sub>4</sub>·5H<sub>2</sub>O</b>	0.53	1.59	1.60
<b>ZnSO<sub>4</sub>·7H<sub>2</sub>O</b>	284.8	854.4	863.00
<b>Selenite-2Na</b>	5.7	17.1	17.30
<b>Ferric Citrate</b>	381.2	1143.6	1155.10

**TABLE B3: Trace Metals 2, 1000X**

COMPONENT	Final Concentration Added (0.33X)	Final Concentration Added (1X)	Liquid 1000X
	µg/L	µg/L	mg/L
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	0.40	1.20	1.20
$\text{AgNO}_3$	0.056	0.168	0.17
$\text{Ba}(\text{C}_2\text{H}_3\text{O}_2)_2$	0.842	2.53	2.55
KBr	0.040	0.12	0.12
$\text{CdCl}_2$	0.75	2.25	2.28
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.785	2.355	2.38
$\text{CrCl}_3$ (anhydrous)	0.015	0.045	0.32
NaF	1.39	4.17	4.20
$\text{GeO}_2$	0.175	0.525	0.53
KI	0.056	0.168	0.17
RbCl	0.400	1.20	1.21
$\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$	1.06	3.18	3.22

**TABLE B4: Trace Metals 3, 1000X**

COMPONENT	Final Concentration Added (0.33X)	Final Concentration Added (1X)	Liquid 1000X
	µg/L	µg/L	mg/L
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	0.056	0.168	0.17
$\text{NaSiO}_3 \cdot 9\text{H}_2\text{O}$	46.2	138.6	140.00
Molybdic Acid, Ammonium Salts	0.409	1.227	1.24
$\text{NH}_4 \text{VO}_3$	0.21	0.63	0.65
$\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$	0.043	0.129	0.13
$\text{SnCl}_2$ (anhydrous)	0.040	0.120	0.12

In this experiment, a chemically defined commercial medium, CD-hybridoma, from Gibco/Life Technology was used as a reference medium. A semi-batch growth profile (a 75% media change was performed daily after Day 3 of the experiment) was initiated to determine the effects of various additives on CDM media. Data at Day 5 were used for this comparison.

**Table I : Comparing the effects of various additives on CDM w/o PAE in a Semi-Batch Growth Profile of C463A.**

<b>Cultures</b>	<b>Media Identification</b>	<b>Viable Cell Density (e/mL)</b>	<b>Total Cell Density (e/mL)</b>	<b>% Viability</b>
<b>A</b>	CDM w/o OPI (oxaloacetate, pyruvate and insulin), bovine transferrin or ferric citrate and defined lipids, trace elements and vitamins	3.24	5.39	60
<b>B</b>	CDM in A above + OPI (oxaloacetate, pyruvate and insulin)	1.14	1.90	60
<b>C</b>	CDM in A above + bovine transferrin or ferric citrate and defined lipids	3.32	4.68	71
<b>D</b>	CDM in A above + trace elements and vitamins ( CDM )	5.22	6.54	80
<b>E</b>	CDM in A above +OPI +transferrin or ferric citrate and defined lipids	1.68	2.26	74
<b>F</b>	CDM in A above + OPI +transferrin or ferric citrate +lipids +trace elements +vitamins	2.74	3.74	73
<b>G</b>	CDM in A above + 1% Sigma PF5F	3.6	4.72	76
<b>H</b>	LTI's CD Hybridoma media-modified	2.64	3.84	69
<b>I</b>	LTI's CD Hybridoma media-modified	3.34	5.04	66

**CDM Medium Performs Best Compared to Other Tested Chemically Defined Media**

Another semi-batch growth profile experiment was initiated to compare the growth performance of C463A in CDM medium to other commercial chemically defined media. Day 3 and subsequent media change is similar to that stated in Table I.

Table II shows the results collected on Day 5 of the semi-batch experiment. The CDM culture reached the highest viable and total densities among the group. C463A viability in CDM medium was also the highest of the four cultures at 82 %. The outcome of this experiment reveals that CDM medium still provides the best support for C463A growth.

**Table II : Comparing CDM to other chemically defined commercial media in a semi-batch growth profile of C463A**

Cultures	Media Identification	Viable Cell Density (e/mL)	Total Cell Density (e/mL)	% Viability
A	CDHY + Sigma S8284 PFSF (50:50)	2.47	3.45	72
B	CDHY + trace elements +vitamins	1.58	3.45	46
C	CDM including + trace elements +vitamins ( CDM )	3.86	4.71	82
D	LTI's CD Hybridoma media-modified	1.52	4.03	38

### **CDM Medium supports high cell density growth and IgG production of C168O, a Remicade producing cell line.**

Once CDM medium was determined to enhance growth in our new host cell line, a semi-batch experiment in spinners was initiated for C168O, a Remicade producing cell line derived from C168J (see, e.g., . Figure A shows that CDM can support high cell density up to  $4.5 \times 10^6$  cells/mL on Day 7. In Figure B, specific productivity for CDM culture is at  $16\mu\text{g}/10^6$  cells/day. Figure C shows that at high cell density between  $4\text{-}5 \times 10^6$  cells/mL, IgG production reached above  $60\text{ }\mu\text{g/mL}$ . Viability of the CDM spinner culture remained above 75% throughout the experiment as seen in Figure D.

### **Quick Adaptation in CDM Medium**

Previously, the adaptation of myeloma cell lines to defined media has been difficult and may take up to 1 year to complete. With CDM medium, the adaptation period has decreased to several weeks. Below, Table III shows another IgG-producing cell line, C380C, adapted to CDM medium in a short period of about 4-5 weeks. After C380C sustained stability in CDM medium, viability was maintained above 90% and doubling time remained within 30-35hrs. The specific productivity and overgrown IgG titer of C380C in CDM are above that when grown in IMDM w/ 5% FBS medium.

**Table III : Adaptation of C380C culture in CDM medium**

Media	Viability	Mean Doubling Time	Specific Productivity (ug/e/day)	Spent culture IgG titer (ug/mL)
IMDM w/ 5% FBS	> 90%	~ 22-24 hrs.	12-13	55-95
CDM	> 90%	Now: 30-40hrs.	17-22	75-140

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It will be clear that the invention can be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.